Wnt Signaling and the Evolution of Embryonic Posterior Development

Minireview

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During vertebrate embryogenesis, most of the meso-dermal tissue posterior to the head forms from a progenitor population that continuously adds blocks of muscles (the somites) from the back end of the embryo. Recent work in less commonly studied arthropods — the flour beetle *Tribolium* and the common house spider — provides evidence suggesting that this posterior growth process might be evolutionarily conserved, with canonical Wnt signaling playing a key role in vertebrates and inverte-brates. We discuss these findings as well as other evidence that suggests that the genetic network control-ling posterior growth was already present in the last common ancestor of the Bilateria. We also highlight other interesting commonalities as well as differences between posterior growth in vertebrates and inverte-brates, suggest future areas of research, and hypothesize that posterior growth may facilitate evolution of animal body plans.

Comparison of body plans across the animal kingdom was an essential exercise leading to Darwin's theory of evolution, and continues to be a central aspect of biological study. In the molecular era, developmental biologists possess a partic-ularly insightful view of this process, as we begin to under-stand how the genome is translated into adult form through the process of embryogenesis. In vertebrates, a major feature of early body development is posterior growth, which accounts for the formation of most of the body posterior to the head. Posterior growth is evident as the addition of segmented blocks of muscle tissue (somites) in an anterior to posterior fashion, as well as the growth of the spinal cord that forms between the somites. Posterior growth is accom-plished by a group of progenitor cells located in a growth zone at the posterior-most end of the embryo, which continuously provides cells to the growing body [1]. Posterior growth contributes to the vast morphological diversity among verte-brate species. The long slender body of a corn snake can grow to over 2 meters in length with over 300 segments, whereas zebrafish have variably 30–35 segments and reach a final size of 5 cm [2]. Recent data from non-model invertebrate species indicate that posterior growth is a common feature of body formation throughout the animal kingdom. In this review, we will discuss the possibility that a conserved molecular pathway governs posterior growth in all animals.

A Wnt–Caudal Pathway for Posterior Growth
The canonical Wnt signaling pathway is an essential devel-opmental regulator that can be found in all extant taxa of metazoans [3]. In vertebrates, Wnt signaling has an early role in establishing the anterior-posterior axis [4], and is then later critically required for posterior growth [5]. In

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both mouse and zebrafish, loss of Wnt signaling results in a severely truncated body, which forms only the head and anterior part of the trunk. The expression of Wnt signaling components in progenitor cells of the growth zone in other vertebrates, such as frog and chicken, suggests that this mechanism is utilized for body plan development by all vertebrates. The Wnt pathway exerts its effects on posterior growth at least in part by directly regulating the expression of the transcription factor Caudal, which in turn activates a suite of *hox* genes expressed in the posterior of the embryo. Like loss of Wnt pathway function, loss of Caudal in mouse and zebrafish results in embryos having only a head and anterior trunk [6,7]. Two papers published recently in *Current Biology* [8,9] indicate that posterior growth through the Wnt–Caudal pathway is conserved in insects and spiders, leading to the intriguing hypothesis that this genetic network is an ancient mechanism of body formation.

A Comparative Look at Posterior Growth — the Importance of Non-Model Organisms
The majority of animals living today are classified as bilater-ians, having both anterior-posterior (head–tail) and dorsal-ventral (back–belly) axes. The bilaterians are divided into three groups, Lophotrochozoa, Ecdysozoa, and Deuterosto-mia (Figure 1). Comparing developmental modes between animals of each group is important for understanding how animal body plans evolved. Traditional comparisons of the molecular control of development have been conducted with genetic model systems, such as the fruit fly *Drosophila melanogaster* and the nematode worm *Caenorhabditis ele-gans*, both belonging to the Ecdysozoa. But we now realize that these animals undergo derived modes of development and thus are perhaps not ideal for evolutionary comparisons (Box 1). *Drosophila*, for example, belongs to the long germ-band insects, which completely lack a posterior growth zone and form all of their segments simultaneously [10,11]. A better evolutionary comparison is drawn using short germ-band insects, which display a more basal mode of arthropod development [10,11] that is used by the vast majority of insects orders. Short germ-band insects have a posterior growth zone and form posterior segments in an anterior to posterior progression [10,11]. In fact, on a gross morphological level, the short germ-band mode of posterior growth is strikingly similar to that of vertebrates (Figure 2).

The fact that posterior growth and segmentation look similar between short germ-band insects and vertebrates does not necessarily indicate that they are evolutionarily homologous. There are many instances throughout the animal kingdom of convergent evolution, where two struc-tures that look similar or serve the same function were achieved by different means [12]. One way to strengthen the argument of homology is to compare the genetic network underlying the development of two similar structures. If the posterior growth zones of insects utilize the same genes as vertebrates, the Wnt pathway and Caudal, they are more likely to be the result of homologous processes [13].
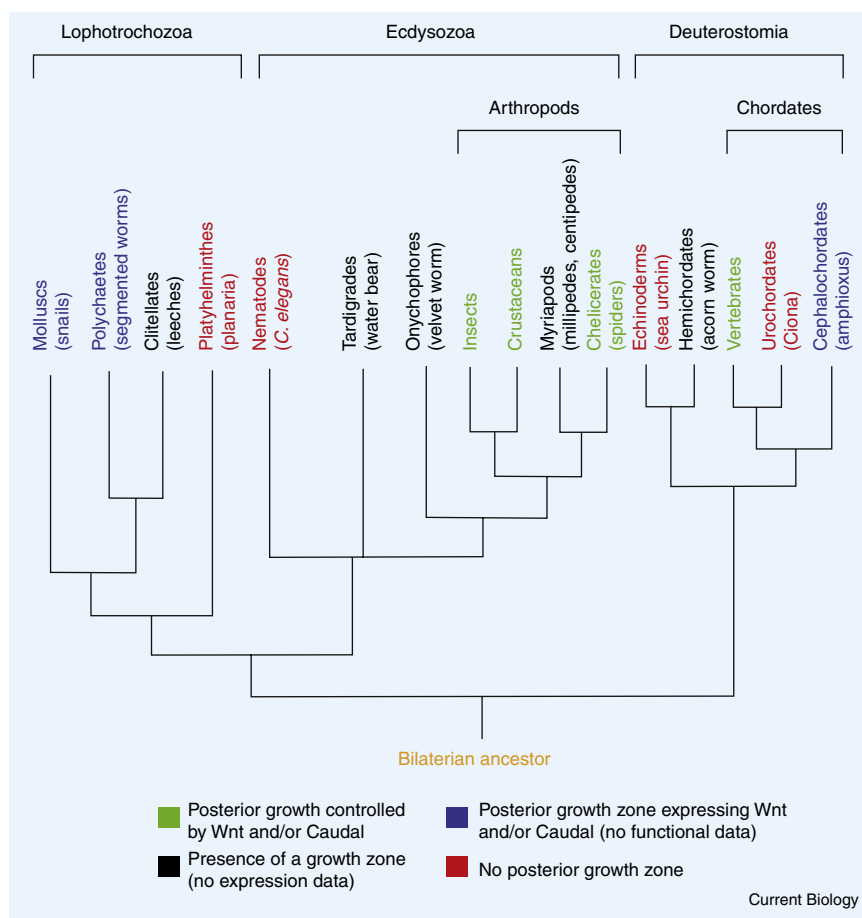
Figure 1. Evidence of posterior growth among the bilaterians.

A phylogenetic tree illustrating the evolutionary relationship between members of the three bilaterian groups, and highlighting our current knowledge of posterior growth in each clade (only clades discussed in the text are illustrated). The presence of posterior growth in deuterostomes and ecdysozoans could mean that it either evolved independently in both lineages or that the common bilaterian ancestor already showed posterior growth. The molecular similarity of posterior growth mechanisms argues for the latter.

Wnt ligands involved in posterior growth have not been identified, loss of β-catenin — the downstream effector of canonical Wnt signaling — causes severe posterior truncations [17]. Moreover, Caudal functions downstream of β-catenin and is required for posterior growth, confirming the genetic hierarchy seen in vertebrates [18].

Spiders are members of the chelicerates, which are grouped together with insects in the arthropod clade (Figure 1). In the common house spider, eliminating expression of a single *wnt* gene caused posterior truncation, as well as an absence of *caudal* expression [9]. In another arthropod clade, the crustaceans, the activity of Caudal was shown to be required for the posterior growth of *Artemia* [16]. Together, these results indicate that posterior growth regulated by the Wnt–Caudal pathway is highly conserved among arthropods and vertebrates.

Homologous Posterior Growth between Vertebrates and Arthropods

Until very recently, loss of function of the Wnt signaling pathway in short germ-band insects had not been examined, making a definitive justification for posterior growth homology difficult. An exciting result clearing the way for this conclusion was recently obtained in the red flour beetle *Tribolium castaneum*. Like vertebrates, the posterior growth zone of the short germ-band *Tribolium* also expresses *wnts* and *caudal* [14,15]. Eliminating the expression of two *wnt* genes with RNA interference caused severe posterior truncations resulting from a failure to maintain the posterior growth zone [8]. While it is not known whether *caudal* is a direct target of Wnt signaling in *Tribolium*, loss of Caudal function causes severe posterior truncation, suggesting that the genetic pathway regulating posterior growth is conserved between vertebrates and short germ-band insects [16].

Further support for evolutionary conservation of posterior growth among insects comes from crickets. While specific

What about the Other Ecdysozoan and Deuterostome Clades?

We have thus far skipped the non-arthropod Ecdysozoa and the non-vertebrate deuterostomes as a matter of necessity rather than convenience. Molecular data needed to draw comparisons to other species simply do not yet exist. For example, other ecdysozoan taxa, such as Tardigrada and Onychophora, display clear posterior growth and segmentation [21], but *wnt* and *caudal* gene expression or function have not been examined (Figure 1).

What about posterior growth in other deuterostome clades? There are two other chordate clades besides the vertebrates, the urochordates and the cephalochordates (Figure 1) [19]. The ascidian *Ciona* is an emerging model

Box 1.

The importance of 'derived' vs. 'basal'.

The terms 'derived' and 'basal' refer to the evolutionary state of a taxon or trait relative to its presumed ancestor. If an animal is significantly different than its ancestor, it is said to be derived, while one that is similar is considered basal. Importantly, derived traits, which make an animal different than its ancestor, can arise by either acquiring new characteristics or losing ancestral traits. When making evolutionary comparisons, it is more informative to use basal animals as they are representative of the ancestral state.

Figure 2. Posterior growth in vertebrates and short germ-band insects.

A comparison of posterior growth in vertebrate and short germ-band insects highlights the striking similarity of this process between these distantly related clades. In vertebrates, the paired somites are blocks of mesoderm that produce muscle, cartilage and bone whereas the ectoderm is unsegmented. In insects, the segments contain both ectoderm and paired somites that produce a number of mesodermal derivatives.


system within the urochordates, but like *Drosophila*, it is considered highly derived, and does not contain a posterior growth zone. Amphioxus on the other hand, a member of the cephalochordates, clearly undergoes posterior growth and somitogenesis. Wnt ligands are expressed in the posterior growth zone, but loss of function studies have not yet been performed [20]. Outside of the chordates, the other major groups within the deuterostomes are the hemichordates and echinoderms. Posterior growth occurs in hemichordates (and arguably in echinoderms), but in morphological terms, does not closely resemble vertebrate or insect posterior growth [21]. It is also not known whether in these groups *wnts* and *caudal* are expressed in the regions where posterior growth is occurring.

The Enigmatic Lophotrochozoa
The fact that the regulation of posterior growth is conserved between insects and vertebrates suggests that their last common ancestor also displayed posterior growth controlled by Wnt signaling and Caudal. Based on modern bilaterian phylogenies, the last common ancestor of insects and vertebrates was also the last common ancestor of all bilaterians [22] (Figure 1). We should, therefore, be able to detect posterior growth in Lophotrochozoan embryos. In terms of their developmental genetics, the Lophotrochozoa are by far the least-studied group among the three major bilaterian clades. Emerging data from the Lophotrochozoa indicate that Wnts and Caudal are present in a posterior growth zone (Figure 1), suggesting that the mechanism of Wnt-controlled posterior growth is also conserved among this group. However, the definitive functional experiments have yet to be done in Lophotrochozoa, and represent an essential missing piece to our overall view of bilaterian posterior growth.

A Chordate Innovation for Wnt-Controlled Posterior Growth
Thus far, we have focused on the similarities of the posterior growth pathway between various bilaterians, but importantly, derivations of posterior growth can also involve novel lineage specific modifications to the core regulatory program. Wnt signaling appears to be at the top of the hierarchy of posterior growth zone regulation in vertebrates. The precise timing of
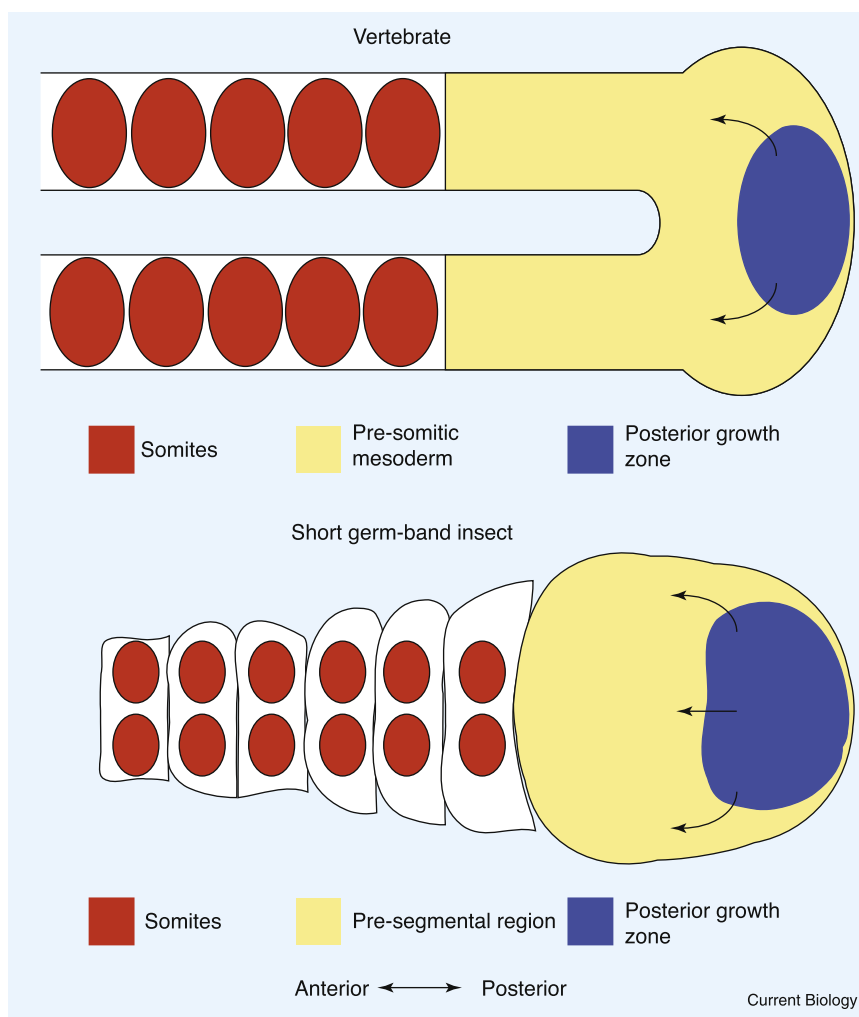
wnt expression in the growth zone during development can control the length of the animal. In zebrafish, artificially inhibiting the Wnt pathway at various times during posterior growth results in embryos with varying length and segment number. The earlier Wnt signaling is inhibited, the shorter the embryo and the fewer the segments [5]. This suggests that in an animal, such as a snake, which forms a large number of segments over many days [2], Wnt signaling is maintained at the posterior end for a very long time.

A direct downstream target of Wnt signaling in vertebrates is *brachyury*, a transcription factor that is itself required for posterior growth [23,24]. We recently showed in zebrafish that the primary role of Brachyury during posterior growth is to directly regulate *wnt* expression, thereby creating a positive feedback loop [5]. In the absence of Brachyury, Wnt signaling is initiated but not maintained, and posterior growth ceases. Interestingly, a *brachyury* homologue is also expressed in the posterior growth zone of *Tribolium* and crickets, although it is not required for posterior growth, indicating that it does not regulate Wnt signaling in insects [25,26]. This raises the intriguing possibility that Brachyury, as it was already expressed in the right location, was co-opted into the posterior growth genetic network along the deuterostome lineage, perhaps to prolong the amount of time Wnt signaling is active in the posterior growth zone
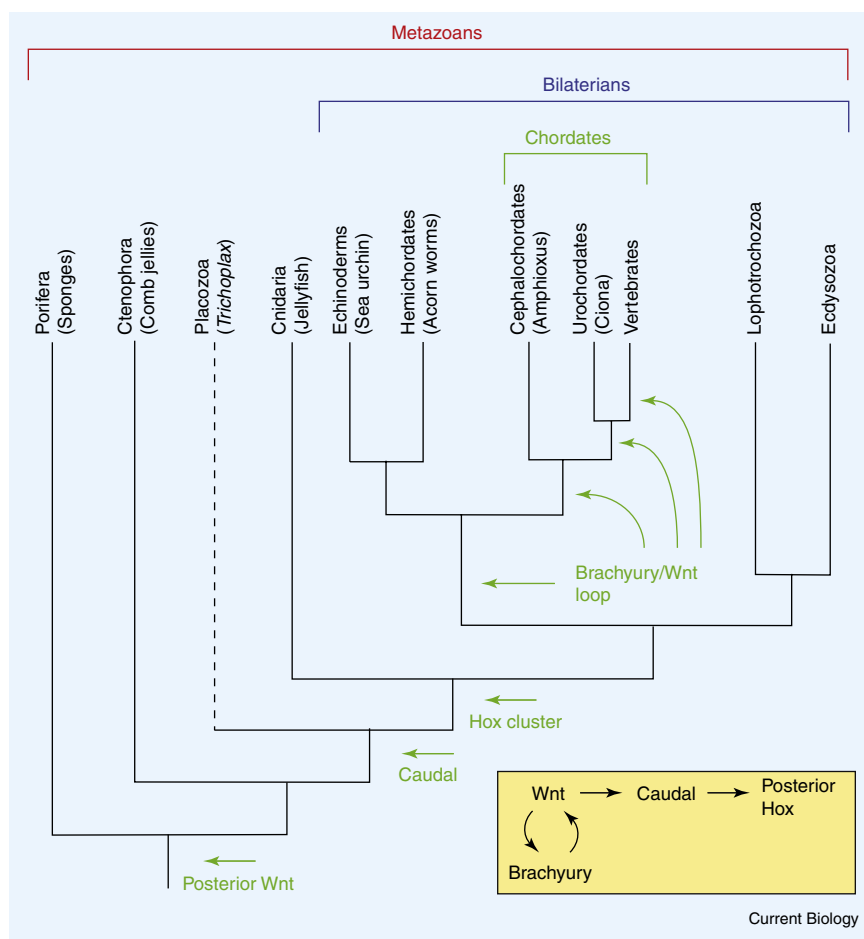
Figure 3. The emergence of the posterior growth pathway.

The essential genetic components of the posterior growth pathway, including the recent innovation of a Brachyury–Wnt loop, are shown in the yellow box. The first appearance of these genetic components during metazoan evolution is indicated on the phylogenetic tree. The dotted line indicates uncertainly in phylogenetic placement of the Placozoa.

segmentation, we favor the idea that the urbilaterian exhibited posterior growth, an idea which has been previously raised [21]. Given the association of Wnt controlled posterior growth with segmentation, and the possibility that they are obligatorily linked, the emergence of posterior growth in the urbilaterian may have required the simultaneous acquisition of segmentation. Coordination of posterior growth may have been simplified by the process of adding serially repeating blocks of tissue, rather than extending an already complex body. The emergence of posterior growth, and possibly segmentation, in the urbilaterian may also have been a necessary prerequisite to the Cambrian explosion, where the fossil record exhibits a sudden burst in morphological diversity among animals. Extending the body axis posteriorly may have provided a mechanism for body plan diversification, which could be subsequently modified to fit specific ecological needs. Extant clades showing clear posterior growth and segmentation, such as the vertebrates, arthropods and annelids, exhibit a disproportionate amount of morphological diversity compared to clades lacking posterior growth.

in order to allow prolonged posterior growth. At which point in animal evolution the Brachyury–Wnt loop was established remains to be determined, although clearly it was present along the vertebrate lineage (Figure 3).

Wnt-Controlled Posterior Growth in Unsegmented Animals?

The animals in which posterior growth has been shown to be Wnt dependent — the vertebrates and arthropods — are clearly segmented. In vertebrates, the posteriorly localized Wnt signal has also been implicated in coordinating the segmentation process [27]. In addition, other groups with overt segmentation, such as cephalochordates and polychaetes, have posteriorly expressed *wnt* [20,28,29], although it is not known whether growth and segmentation are coordinated by Wnts in these animals. The association with Wnt-controlled posterior growth and segmentation (working through the Notch pathway) raises the possibility that these two processes are intricately, and perhaps obligatorily, linked. It is clear that segmentation can be achieved without posterior growth, as in the case of *Drosophila*, but whether posterior growth can occur without segmentation is unknown. The molluscs, which exhibit posterior growth but are not segmented, provide a unique opportunity to examine this question. Currently, however, *wnt* expression during posterior growth in molluscs has not been examined.

Arguments have been made for and against the hypothesis that the common bilaterian ancestor, the so-called urbilaterian, was segmented [30,31]. Regardless of the state of

Evolutionary Origins of a Wnt–Caudal Posterior Growth Mechanism

All bilaterians have *hox* genes, which are transcription factors that specify regional identity along the anterior-posterior axis [30]. Anteriorly expressed *hox* genes specify the head, while posteriorly expressed *hox* genes specify the back end. In vertebrates and arthropods (and likely all bilaterians), Caudal activates the expression of posterior *hox* genes, thereby imparting posterior identity to regions in which it is expressed. Using this Wnt–Caudal–Hox hierarchy, we can attempt to trace back the origins of posterior growth based on when these genes first appear in metazoan evolution (Figure 3).

There are four metazoan phyla outside of the bilaterians, Porifera (sponges), Ctenophora (comb jellies), Placozoa (*Trichoplax*), and Cnidaria (sea anemones, corals, jellyfish). There are no clear examples of posterior growth among these groups, despite the fact that all of them contain Wnts [32]. Sponges, which potentially represent the evolutionarily oldest branch of the metazoan phylogeny, have been shown to have posteriorly localized *wnt* expression in their larvae, the role of which is unknown [33]. During evolution, this posteriorly localized Wnt signal could have been co-opted into

a new role of Caudal regulation. Caudal does not appear in evolution until the emergence of Placozoa, and finally *hox* clusters appear in Cnidaria [3]. While a *hox* cluster is present in Cnidarians, it lacks the full complement of trunk *hox* genes seen in bilaterians [21]. Posterior growth may require these additional genes to specify an extended body axis. Based on the comparative data discussed earlier, combined with the ancestry of the genes involved in posterior growth, this process of body plan development is likely to have evolved at or around the emergence of the urbilaterian. A further refinement then occurred somewhere along the deuterostome lineage as discussed above, imparting a Wnt–Brachyury loop to sustain posterior growth (Figure 3).

Future Directions

The studies discussed in this review have provided the outline of a possible ancestral mechanism for the molecular regulation of posterior growth, and have also opened up many new questions to pursue. In the variety of non-model organisms that show posterior growth, it will be extremely valuable to determine if they express *wnts* and *caudal*, and important to investigate whether inhibition of the function of these genes leads to abnormalities in posterior growth. With the increasing availability of genome information for these organisms, as well as the development of loss-of-function tools, such as morpholinos and RNAi, these types of studies are now feasible. In vertebrates, *caudal* is clearly a direct target of Wnt signaling, but this has yet to be established for other organisms. Demonstrating direct regulation of the *caudal* promoter in invertebrate systems by Wnt signaling would be a valuable addition in establishing homology of mechanism. Similarly, examining other deuterostomes, particularly the basal chordate amphioxus, would help establish when the Wnt–Brachyury loop appeared in development.

While we have focused on Wnt as a regulator of *caudal* expression, Wnt signaling can activate a plethora of genes depending on the cellular context, including genes involved in cell growth, segmentation and embryonic patterning. In the years to come, identifying which Wnt targets are activated in the posterior growth zone of different bilaterians will be essential for understanding which aspects of posterior growth are conserved, and which have undergone change to create the diversity we see today.

References

1. Holley, S.A. (2007). The genetics and embryology of zebrafish metamerism. Dev. Dyn. *236*, 1422–1449.
2. Gomez, C., Ozbudak, E.M., Wunderlich, J., Baumann, D., Lewis, J., and Pourquie, O. (2008). Control of segment number in vertebrate embryos. Nature *454*, 335–339.
3. Ryan, J.F., and Baxevanis, A.D. (2007). Hox, Wnt, and the evolution of the primary body axis: insights from the early-divergent phyla. Biol. Direct. *2*, 37.
4. Schier, A.F., and Talbot, W.S. (2005). Molecular genetics of axis formation in zebrafish. Annu. Rev. Genet. *39*, 561–613.
5. Martin, B.L., and Kimelman, D. (2008). Regulation of canonical Wnt signaling by Brachyury is essential for posterior mesoderm formation. Dev. Cell *15*, 121–133.
6. Chawengsaksophak, K., de Graaff, W., Rossant, J., Deschamps, J., and Beck, F. (2004). *Cdx2* is essential for axial elongation in mouse development. Proc. Natl. Acad. Sci. USA *101*, 7641–7645.
7. Shimizu, T., Bae, Y.K., Muraoka, O., and Hibi, M. (2005). Interaction of Wnt and *caudal*-related genes in zebrafish posterior body formation. Dev. Biol. *279*, 125–141.
8. Bolognesi, R., Farzana, L., Fischer, T.D., and Brown, S.J. (2008). Multiple *Wnt* genes are required for segmentation in the short-germ embryo of *Tribolium castaneum*. Curr. Biol. *18*, 1624–1629.
9. McGregor, A.P., Pechmann, M., Schwager, E.E., Feitosa, N.M., Kruck, S., Aranda, M., and Damen, W.G. (2008). *Wnt8* is required for growth-zone establishment and development of opisthosomal segments in a spider. Curr. Biol. *18*, 1619–1623.
10. Liu, P.Z., and Kaufman, T.C. (2005). Short and long germ segmentation: unanswered questions in the evolution of a developmental mode. Evol. Dev. *7*, 629–646.
11. Rosenberg, M.I., Lynch, J.A., and Desplan, C. (2008). Heads and tails: Evolution of antero-posterior patterning in insects. Biochim. Biophys. Acta, epub ahead of print.
12. Wake, D.B. (1991). Homoplasy: the result of natural selection, or evidence of design limitations? Am. Nat. *138*, 543–567.
13. Abouheif, E. (1999). Establishing homology criteria for regulatory gene networks: prospects and challenges. Novartis Found. Symp. *222*, 207–221, discussion 222–205.
14. Schulz, C., Schroder, R., Hausdorf, B., Wolff, C., and Tautz, D. (1998). A *caudal* homologue in the short germ-band beetle *Tribolium* shows similarities to both, the *Drosophila* and the vertebrate *caudal* expression patterns. Dev. Genes. Evol. *208*, 283–289.
15. Bolognesi, R., Beermann, A., Farzana, L., Wittkopp, N., Lutz, R., Balavoine, G., Brown, S.J., and Schroder, R. (2008). *Tribolium Wnts*: evidence for a larger repertoire in insects with overlapping expression patterns that suggest multiple redundant functions in embryogenesis. Dev. Genes. Evol. *218*, 193–202.
16. Copf, T., Schroder, R., and Averof, M. (2004). Ancestral role of *caudal* genes in axis elongation and segmentation. Proc. Natl. Acad. Sci. USA *101*, 17711–17715.
17. Miyawaki, K., Mito, T., Sarashina, I., Zhang, H., Shinmyo, Y., Ohuchi, H., and Noji, S. (2004). Involvement of Wingless/Armadillo signaling in the posterior sequential segmentation in the cricket, *Gryllus bimaculatus* (Orthoptera), as revealed by RNAi analysis. Mech. Dev. *121*, 119–130.
18. Shinmyo, Y., Mito, T., Matsushita, T., Sarashina, I., Miyawaki, K., Ohuchi, H., and Noji, S. (2005). *caudal* is required for gnathal and thoracic patterning and for posterior elongation in the intermediate-germband cricket *Gryllus bimaculatus*. Mech. Dev. *122*, 231–239.
19. Swalla, B.J., and Smith, A.B. (2008). Deciphering deuterostome phylogeny: molecular, morphological and palaeontological perspectives. Philos. Trans. R. Soc. Lond. B Biol. Sci. *363*, 1557–1568.
20. Schubert, M., Holland, L.Z., Stokes, M.D., and Holland, N.D. (2001). Three amphioxus *Wnt* genes (*AmphiWnt3*, *AmphiWnt5*, and *AmphiWnt6*) associated with the tail bud: the evolution of somitogenesis in chordates. Dev. Biol. *240*, 262–273.
21. Jacobs, D.K., Hughes, N.C., Fitz-Gibbon, S.T., and Winchell, C.J. (2005). Terminal addition, the Cambrian radiation and the Phanerozoic evolution of bilaterian form. Evol. Dev. *7*, 498–514.
22. Dunn, C.W., Hejnol, A., Matus, D.Q., Pang, K., Browne, W.E., Smith, S.A., Seaver, E., Rouse, G.W., Obst, M., Edgecombe, G.D., *et al.* (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. Nature *452*, 745–749.
23. Arnold, S.J., Stappert, J., Bauer, A., Kispert, A., Herrmann, B.G., and Kemler, R. (2000). *Brachyury* is a target gene of the Wnt/β-catenin signaling pathway. Mech. Dev. *91*, 249–258.
24. Yamaguchi, T.P., Takada, S., Yoshikawa, Y., Wu, N., and McMahon, A.P. (1999). *T (Brachyury)* is a direct target of Wnt3a during paraxial mesoderm specification. Genes Dev. *13*, 3185–3190.
25. Berns, N., Kusch, T., Schroder, R., and Reuter, R. (2008). Expression, function and regulation of *Brachyenteron* in the short germband insect *Tribolium castaneum*. Dev. Genes Evol. *218*, 169–179.
26. Shinmyo, Y., Mito, T., Uda, T., Nakamura, T., Miyawaki, K., Ohuchi, H., and Noji, S. (2006). *brachyenteron* is necessary for morphogenesis of the posterior gut but not for anteroposterior axial elongation from the posterior growth zone in the intermediate-germband cricket *Gryllus bimaculatus*. Development *133*, 4539–4547.
27. Dequeant, M.L., and Pourquie, O. (2008). Segmental patterning of the vertebrate embryonic axis. Nat. Rev. Genet. *9*, 370–382.
28. Schubert, M., Holland, L.Z., Panopoulou, G.D., Lehrach, H., and Holland, N.D. (2000). Characterization of amphioxus *AmphiWnt8*: insights into the evolution of patterning of the embryonic dorsoventral axis. Evol. Dev. *2*, 85–92.
29. Seaver, E.C., and Kaneshige, L.M. (2006). Expression of 'segmentation' genes during larval and juvenile development in the polychaetes *Capitella* sp. I and *H. elegans*. Dev. Biol. *289*, 179–194.
30. De Robertis, E.M. (2008). Evo-devo: variations on ancestral themes. Cell *132*, 185–195.
31. Rivera, A.S., and Weisblat, D.A. (2009). And Lophotrochozoa makes three: Notch/Hes signaling in annelid segmentation. Dev. Genes Evol. *219*, 37–43.
32. Nichols, S.A., Dirks, W., Pearse, J.S., and King, N. (2006). Early evolution of animal cell signaling and adhesion genes. Proc. Natl. Acad. Sci. USA *103*, 12451–12456.
33. Adamska, M., Degnan, S.M., Green, K.M., Adamski, M., Craigie, A., Larroux, C., and Degnan, B.M. (2007). Wnt and TGF-β expression in the sponge *Amphimedon queenslandica* and the origin of metazoan embryonic patterning. PLoS ONE *2*, e1031.